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Reversal of Photoschedule in Spring Does not Prevent Photorefractoriness in Siberian Hamsters

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ABSTRACT We studied the influence of light–dark (L:D) cycle reversal on daily variations in the brown adipose tissue (BAT) capacity for nonshivering thermogenesis (NST) in Siberian hamsters (*Phodopus sungorus*). Continuous and simultaneous measurements of BAT temperature (T_{BAT}) and preferred ambient temperature (PT_a) were made after noradrenaline (NA) injections administered every 4 hr. First, hamsters were acclimated for 4 weeks to an ambient temperature (T_a) of 23°C and 12L:12D, and then to a reversed photoschedule 12D:12L for 8 weeks. The same was done after a 4- and 8-week acclimation period at the same T_a. We found that after photoschedule reversal, the re-entrainment of T_{BAT} and PT_a rhythms preceded re-entrainment of the NST rhythm. The daily rhythms of T_{BAT} and PT_a were fully re-entrained after 4 weeks of acclimation to the reversed photoschedule, but rhythmicity of the response to NA disappeared. This rhythm was restored in hamsters acclimated to a reversed photoschedule for 8 weeks. We suggest that the daily rhythm of NST capacity is not responsible for generating the rhythm of body temperature (T_b). Rather, it is a result of the daily rhythm of T_b, but adjusts to the new environment more slowly than the T_b rhythm. When a daily rhythm of NST was present, the increase in T_{BAT} after NA injection was inversely correlated with the pre-injection T_{BAT}. In addition, NA-induced changes in PT_a reflected the intensity of NST in BAT; namely, increased T_{BAT} was correlated with the post-injection decrease in PT_a. When the increase in T_{BAT} was large, animals chose a lower T_a to dissipate excessive heat and prevent overheating. In the course of the experiments, we recorded a decreased mean NST capacity and increased body mass of hamsters. These changes are representative of the time of photorefractoriness and a transition to a summer status. Despite prolonged exposure to an intermediate day length (12 hr of light) and photoschedule reversal, hamsters continued to change towards their summer condition and were able to acclimate to the new D:L cycle. *J. Exp. Zool. 303A:976–986, 2005.*

Siberian hamsters (*Phodopus sungorus*) are small nocturnal rodents that exhibit a robust circadian rhythm of body temperature (T_b; Scribner and Wynne-Edwards, '94; Ross, '98; Ruf and Heldmaier, 2000). As in other nocturnal mammals, T_b in hamsters is relatively high at night (i.e., in their active phase, α; see Refinetti and Menaker ('92) for a review). Hamsters become active immediately or shortly after the lights are turned off, while the end of the α phase coincides with lights on. When at rest, hamsters maintain their body temperature at about 36°C. During the active phase, T_b rises to temperatures above 37°C (Puchalski and Lynch, '86; Klante and Steinlechner, '95). An amplitude of daily variations in T_b depends on photoperiod: in hamsters acclimated to a long day, this amplitude is smaller by about 1°C than in hamsters acclimated to a short day (Heldmaier et al., '89; Ruby and Zucker, '92).

The primary pacemaker for the regulation of circadian rhythms is situated in the suprachiasmatic nuclei (SCN) of the hypothalamus. Indeed, after bilateral lesion of the SCN, T_b rhythm disappears (Ruby et al., '89). From the SCN, the photoperiodic information is transferred to the pineal gland and results in the melatonin signal. Regardless of the species' activity pattern (diurnal or nocturnal), the melatonin level is higher during darkness (Saarela and Reiter, '93). The Siberian hamster is a highly photoperiodic rodent that

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strongly depends on changes in photoperiod to cue acclimatization, while ambient temperature has a minor effect (Ruf et al., '93). Under a short photoperiod, T_b is lower than under a long photoperiod, but larger ultradian variations are recorded. Such changes in circadian organization of T_b might allow for energy savings (Heldmaier et al., '85, '89). The reduction of energy requirements is of vital importance in a cold environment, which in the natural environment of hamsters usually coincides with short days.

In autumn, when the photoperiod shortens, hamsters are sensitive to short days (photosensitive) and prepare for winter: they lose body mass, increase nonshivering thermogenesis (NST) capacity, their fur changes to white, and they start to exhibit daily torpor and gonadal regression. In spring (or after 4–5 months under a short photoperiod), hamsters become photorefractory (i.e., insensitive to short days) and spontaneously return to their summer status. During the state of photorefractoriness, hamsters increase body mass, their fur changes to a grey one for summer and they exhibit gonadal recrudescence. Daily torpor is ceased and NST capacity is decreased to a lower, summer level (Hoffmann, '73; Heldmaier and Steinlechner, '81; Heldmaier et al., '81, '82; Heldmaier and Lynch, '86; Lynch and Puchalski, '86; Jefimow et al., 2004b). Seasonal changes in thermoregulation and behaviour ensure winter survival. In the Siberian hamster, as in many other small placental mammals, NST is a primary source of heat and plays a very important role in the maintenance of normothermy (Janský, '73). In hamsters exposed to a short photoperiod, NST capacity is higher than in animals exposed to a long one (Heldmaier et al., '82). In addition, NST capacity also depends on the time of day (Jefimow et al., 2000, 2003). Also, in other rodents, such as spiny mice (*Acomys russatus* and *Acomys cahirinus*) and wood mice (*Apodemus sylvaticus*), daily variations in NST have been recorded (Kronfeld et al., '94; Haim et al., '95; Haim and Zisapel, '99). However, the basis of these variations is not yet well understood.

In small placental mammals, the major site for regulatory NST is brown adipose tissue (BAT; Janský, '73; Nicholls and Locke, '84; Kuroshima, '93). Thus, temperature in the BAT can be used as an indicator of NST capacity (Hayward and Lyman, '67; Hayward, '68; Hashimoto et al., 2002; Jefimow et al., 2004a).

In the present study, we hypothesized that NST capacity depends on the actual level of T_b and is strongly influenced by the prevalent light–dark cycle. We predicted that the rhythm of NST capacity, as related to the T_b rhythm, would follow daily changes in T_b that would occur as a result of adjustment to new photoperiodic conditions. Hence, after acclimation to a reversed photoschedule and re-entrainment of a T_b rhythm, the daily rhythm of BAT capacity for regulatory NST should also be re-entrained. This would bring about higher NST capacity during the resting (ρ) phase and lower NST capacity during the active (α) phase, independent of the photoperiodic regime. In addition, since the daily rhythm of preferred ambient temperature (PT_a) opposes the daily rhythm of T_b (Gordon, '94; Refinetti, '95), we predicted that the daily rhythm of PT_a would follow the re-entrainment of T_b rhythm, being higher when T_b is low and vice versa.

In order to test our predictions, we investigated the influence of T_b (measured as interscapular T_{BAT}) rhythm on daily variations in BAT capacity for regulatory NST in Siberian hamsters acclimated to a 12L:12D photoschedule, and then to a reversed 12D:12L photoschedule. To test the thermogenic capacity of BAT, we administrated noradrenaline (NA) every 4 hr and continuously monitored T_{BAT} and PT_a. Since NA activates NST and induces hyperthermia (Janský, '73), the magnitude of changes in BAT temperature after NA would reflect the intensity of NST. When NST is activated, hamsters would prefer lower ambient temperatures to facilitate the maintenance of normothermic body temperature (Gordon, '93).

MATERIAL AND METHODS

Animals and housing

Nine adult (4-month-old) male Siberian hamsters (*P. sungorus*) from our own breeding colony were used in this study. Before the start of the experiments in March, the animals were housed individually in standard laboratory cages ($33 \times 20 \times 18$ cm) under the natural photoperiod (12L:12D in March). At the start of the first set of experiments, the animals were moved to a climate chamber on a 12L:12D cycle (L:D cycle; lights on at 09:00), with an ambient temperature (T_a) of 23°C for 4 weeks. Food and water were available ad libitum. During the next 4 weeks, the hamsters were housed in the same climate chamber, but with a reversed photoschedule [i.e. 12D:12L (D:L cycle; lights on at 21:00 h)] at a T_a of 23°C. After 8 weeks under the reversed photoschedule, the animals were sampled again. Photoschedule

reversal was accomplished by the prolongation of the light phase of the cycle; that is, on the day of reversal the hamsters experienced 24L:12D.

After acclimation to the L:D cycle, six out of nine animals were used. After 4 weeks of acclimation to D:L cycle, three previously used hamsters were replaced with new ones that were acclimated simultaneously, but were not used in the first set of experiments. This replacement was applied to evaluate whether injections during the first experiment could affect the results of the second one. After 8 weeks of acclimation to D:L cycle, six hamsters used in the previous experiments were used again.

To monitor changes in body mass (m_b), which are a good indicator of acclimation in Siberian hamsters (Hoffmann, '73), they were weighed to ± 0.1 g before experiment and then after acclimation to L:D and D:L cycles (i.e., every 4 weeks).

Measurements of T_{BAT} and PT_a

At least 3 days before an experiment, we implanted a polyethylene cannula (0.8 mm in diameter, 5 cm in length, SIMS Portex Ltd., Hythe, UK) in each hamster under Sevorane (Abbott Laboratories Ltd., Queensborough, UK) anaesthesia. The cannula was inserted under the skin, through a small incision (3 mm) at the back of the neck, and then fixed with surgical thread and adhesive to the skin. During the experiments, the cannula served as a guide for a copper-constantan thermocouple (0.6 mm in diameter, W-TW-36 P2; Physitemp Instruments Inc., Clifton, NJ, USA).

After acclimation to L:D and D:L cycles, each animal was tested individually in a thermal gradient system that allowed for continuous and simultaneous measurements of preferred ambient temperature (PT_a) and BAT temperature (T_{BAT}). The thermal gradient consisted of a long aluminium chamber (120 cm length $\times$ 10 cm height $\times$ 8 cm width), divided by half-width partitions into 16 compartments of the same size, and covered with transparent Perspex to permit light entry. The system was heated at one end and cooled at the other, resulting in a range of temperatures increasing linearly from 5°C to 45°C. The position of the animal, and thus its selected temperature, was detected at 1-sec intervals by infrared photoemitter–photodetector pairs placed in each compartment. A narrow slit in the transparent lid allowed movement of the thermocouple that was suspended above the gradient by an elastic band. This design allowed hamsters to move freely inside the gradient without a load. T_{BAT} and PT_a were automatically recorded at 1-sec intervals and saved on disk.

Experimental design

Response to NA

Each animal was placed in the thermal gradient box for 3 days. On the first day, the thermocouple wire was inserted into the cannula to a depth where large BAT deposits are present. Then the hamster was left undisturbed to enable its habituation. On the next day, beginning at 09:00, NA [(±)-Arterenol; Sigma-Aldrich Chemin GmbH, Steinheim, Germany] at a dose of 0.6 mg kg^{-1} or saline (in the same volume as NA) was injected subcutaneously every 4 hr. Saline injections were a control for NA; while NA activates NST, changes in body temperature after saline reflect stress of injection. Night-time injections were done under dim red light. Half of the animals were injected with NA on the second day and then with saline on the third day. The sequence of injections in the other half of animals was reversed (first day—undisturbed, second day—saline, third day—NA). T_{BAT} and PT_a in saline and NA-injected hamsters were measured continuously. Temperatures before each injection were used to assess daily rhythms of T_{BAT} and PT_a. After acclimation to L:D cycle, injections were administered at 09:00 (CT 0; circadian time 0: beginning of the light phase), 13:00 (CT 4), 17:00 (CT 8), 21:00 (CT 12), 01:00 (CT 16) and 05:00 (CT 20). After acclimation to the reversed photoschedule (i.e., after 4 and 8 weeks in D:L cycle), injections were administered at 09:00 (CT 12), 13:00 (CT 16), 17:00 (CT 20), 21:00 (CT 0), 01:00 (CT 4) and 05:00 (CT 8). While in the thermal gradient, hamsters were offered food and water ad libitum. Eight feeders were placed equidistant along the gradient to avoid the influence of food searching on PT_a. If hamsters chewed their thermocouple lead wires, the break was immediately repaired. During lead repair, animals were handled for no more than 10 min.

Statistical analysis

All recorded data were plotted at 10-min intervals as means ± SE. If a hamster chewed its lead shortly before or after injection, data collected for 1 hr after the repair were discarded. To assess daily rhythms of T_{BAT} and PT_a, data collected 30 min before each injection were analysed. To

assess the effects of saline and NA on T_{BAT} and PT_a, data collected 30 min before (as a reference) and 60 min after each injection were compared. We found that the sequence of injections did not influence T_{BAT} or PT_a (two-way ANOVA, $P = 0.85$). There was no difference in the response to saline or to NA by hamsters that were used for the first time or re-tested (two-way ANOVA, $P = 0.32$). Changes in T_{BAT} and PT_a, induced by injected pharmacological agents, are presented as the difference between mean T_{BAT} or PT_a recorded within 60 min after and 30 min before the injection (ΔT_{BAT}; ΔPT_a). To clarify the results, in the text below, T_{BAT0} refers to BAT temperature before injection and T_{BAT1} refers to BAT temperature after injection.

Changes in T_{BAT0}, T_{BAT1} and PT_a were analysed using three-way ANOVA to investigate the influence of photoschedule, type of injection and time of day. When ANOVA showed significant influence of the analysed factors, a post-hoc LSD test followed by a Bonferroni correction was used for comparison of means. The Pearson correlation coefficient, r, was used to test for correspondence between T_{BAT1} and PT_a as well as between T_{BAT0} and T_{BAT1}. Changes in body mass (m_b) were analysed using one-way ANOVA followed by Tukey's post-hoc test. Differences were considered statistically significant if $P < 0.05$. All values are presented as mean ± SE.

Experiments were approved by the Local Committee for the Ethics in Animal Research.

RESULTS

The effects of 4 weeks of acclimation to the reversed photoschedule

BAT temperature (T_{BAT}; Fig. 1)

After 4 weeks of acclimation to a reversed photoschedule (D:L cycle), the daily rhythm of T_{BAT} before injection (T_{BAT0}) was fully reversed and entrained to a new D:L cycle (Fig. 1). A three-way ANOVA revealed the significant influence of time of day on T_{BAT0} ($F_{(5,101)} = 7.06$, $P < 0.0001$), while type of injection and photoschedule had no effects. The magnitude of changes in T_{BAT} after injection (T_{BAT1}) was significantly correlated with the type of injection, photoschedule and time of injection (three-way ANOVA: $F_{(5,100)} = 2.95$, $P < 0.05$; Fig. 1). In general, NA induced a larger increase in T_{BAT} than saline and this increase was larger in hamsters acclimated to L:D than to D:L cycle.

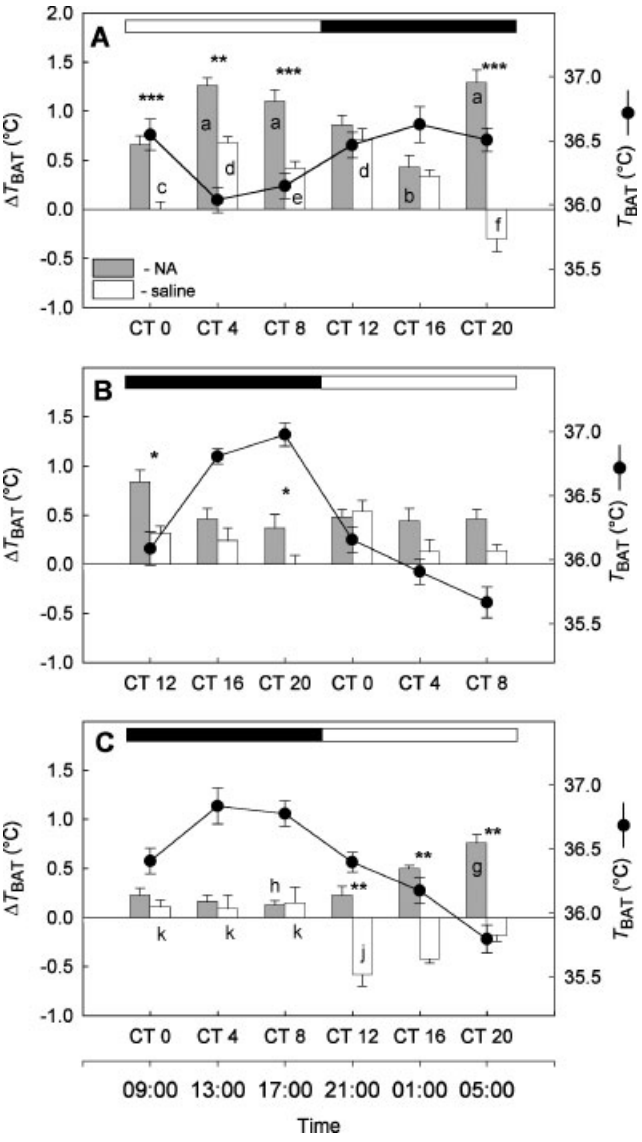

Fig. 1. Mean (±SE) brown adipose tissue temperature (T_{BAT}) before each injection (—●—) and changes in T_{BAT} (ΔT_{BAT}; bars) induced by saline and noradrenaline (NA) injections in Siberian hamsters acclimated for 4 weeks to L:D cycle (panel **A**) and then after 4 and 8 weeks of acclimation to a reversed photoschedule (panels **B** and **C**, respectively). Dark bars at the top of each panel indicate time of darkness. Stars indicate significant differences between saline- and NA-injected hamsters: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Significantly different values within each group are indicated by the superscripts: a–b, c–d, e–f, g–h, j–k: $P < 0.05$; d–f: $P < 0.001$.

After acclimation to L:D cycle, daily variations in the response to NA and saline were also recorded; the largest increases in T_{BAT1} after NA were recorded at CT 4, CT 8 and CT 20 and after saline at CT 4 and CT 12. After 4 weeks of acclimation to a reversed photoschedule (D:L cycle), daily variations in the response to saline or NA injections

disappeared. However, the increase in T_{BAT1} after NA injection at CT 4, CT 8 and CT 20 was smaller than that after acclimation to L:D cycle ($P<0.001$, 0.004 and 0.001, respectively; Fig. 1). In saline-injected hamsters acclimated for 4 weeks to D:L cycle, the increase in T_{BAT1} at CT 4 and CT 12 was smaller than that after acclimation to L:D cycle ($P<0.008$ and 0.05, respectively), while at CT 0 the increase in T_{BAT1} was larger ($P<0.02$).

Preferred ambient temperature (PT_a; Fig. 2)

After 4 weeks of acclimation to D:L cycle, the daily rhythm of preferred ambient temperature was also re-entrained. This temperature was significantly correlated with time of day (three-way ANOVA: $F_{(5,89)}=2.96$, $P<0.05$, Fig. 2) while type of injection and photoschedule were not correlated. The magnitude of changes in PT_a after injection was closely correlated with type of injection, photoschedule and time of day (three-way ANOVA: $F_{(5,92)}=2.45$, $P<0.04$; Fig. 2). NA always caused a more pronounced decrease in PT_a than saline, except for the injection at the end of night (CT 20) in animals acclimated to D:L cycle.

We also recorded daily variations in PT_a after NA. In hamsters acclimated to L:D cycle, the time of day did not significantly affect the decrease in PT_a after NA injection; however, it varied from -4.2 ± 0.5°C to -8.0 ± 1.0°C. After photoschedule reversal, preferred ambient temperature of saline-injected hamsters did not correlate with the time of day, while in NA-injected hamsters, the decrease in PT_a at CT 0, CT 4, CT 8 and CT 12 was larger than at CT 20 (Fig. 2).

The magnitude of changes in preferred ambient temperature depended on photoschedule. After acclimation to L:D cycle, the decrease in PT_a after NA injection at the beginning of the day (CT 0) and the end of night (CT 20) was larger than after acclimation to the reversed photoschedule ($P<0.05$ and 0.001, respectively). In saline-injected hamsters, differences were recorded at CT 12 and CT 20 ($P<0.03$ and 0.02, respectively; Fig. 2).

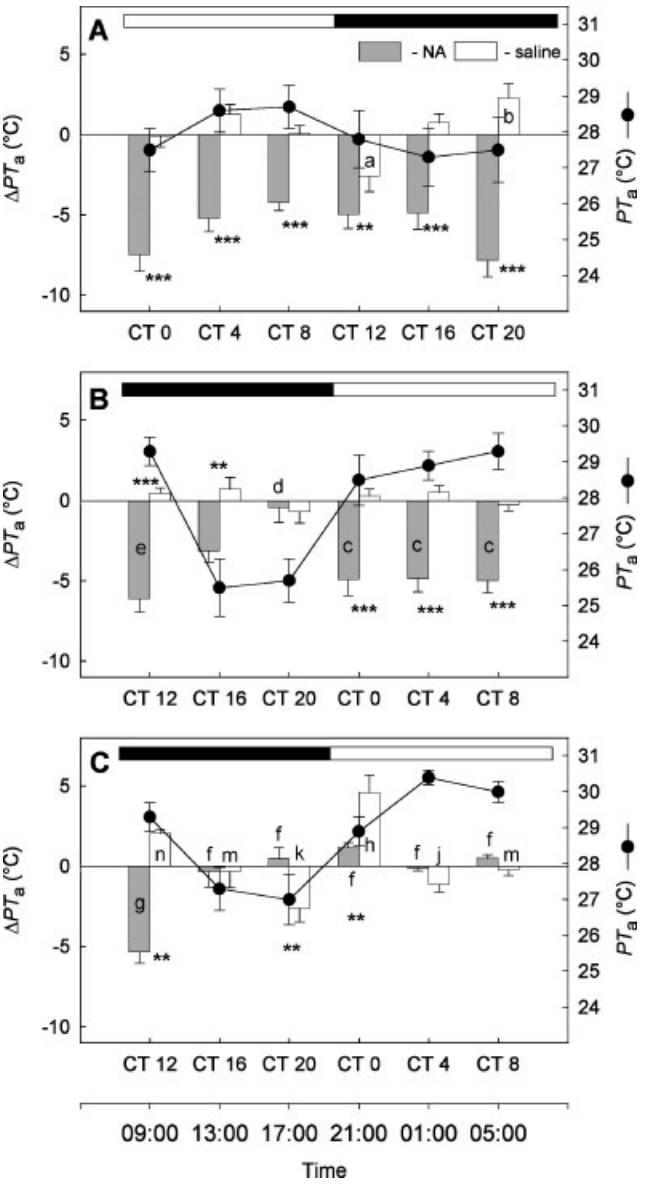

Fig. 2. Mean ($\pm$SE) preferred ambient temperature (PT_a) before each injection (—●—) and changes in PT_a (ΔPT_a; bars) induced by saline and noradrenaline (NA) injections in Siberian hamsters acclimated for four weeks to L:D cycle (panel **A**) and then after 4 and 8 weeks of acclimation to a reversed photoschedule (panels **B** and **C**, respectively). Dark bars at the top of each panel indicate time of darkness. Stars indicate significant differences between saline- and NA-injected hamsters: $^{**}P<0.01$, $^{***}P<0.001$. Significantly different values within each group are indicated by the superscripts: a–b, c–d: $P<0.05$; d–e, f–g, h–j, h–k: $P<0.001$; h–m, k–n: $P<0.01$.

Correlation between T_{BAT0}, T_{BAT1} and (PT_a; Table 1)

In hamsters acclimated to L:D cycle, the magnitude of increase in BAT temperature after injection (T_{BAT1}) was inversely correlated with pre-injection temperature of BAT (T_{BAT0}); the higher the initial temperature, the smaller was the increase after injection. After photoschedule reversal, such a correlation was found only in saline-treated hamsters (Table 1). Before injection, T_{BAT0} was inversely correlated with PT_a only in animals acclimated to D:L cycle ($r=-0.49$,

TABLE 1. The increase in brown adipose tissue temperature (T_{BAT}) after noradrenaline (NA) and saline injections was inversely correlated with T_{BAT} before injection in Siberian hamsters acclimated to L:D cycle and then after 4 and 8 weeks of acclimation to a reversed photoschedule (D:L 4 and D:L 8, respectively)

Photoschedule	Injection	r	P
L:D	NA	−0.56	0.002
	Saline	−0.68	0.001
D:L 4	NA	−0.32	n.s. (0.08)
	Saline	−0.40	0.03
D:L 8	NA	−0.79	0.001
	Saline	−0.62	0.001

Only in NA-injected hamsters acclimated for 4 weeks to D:L cycle, this correlation was not significant.

$P<0.001$). Increase in T_{BAT1} after NA injection was correlated with decreased PT_a, both in animals acclimated to L:D cycle ($r=0.36$, $P<0.001$), as well as to D:L cycle ($r=0.48$, $P<0.001$). In saline-injected hamsters, significant correlation was found only after acclimation to D:L cycle ($r=0.32$, $P<0.001$).

The effects of 8 weeks of acclimation to the reversed photoschedule

BAT temperature (T_{BAT}; Fig. 1)

After 8 weeks of acclimation to the reversed photoschedule, the daily rhythm of T_{BAT0} was also fully reversed in comparison to L:D cycle (three-way ANOVA: $F_{(5,99)}=5.30$, $P<0.001$, Fig. 1). BAT temperatures (T_{BAT0}) in saline- and NA-injected hamsters did not differ. The magnitude of changes in BAT temperature after injection (T_{BAT1}) was significantly correlated with type of injection, photoschedule and time of day (three-way ANOVA: $F_{(5,98)}=2.32$, $P<0.05$; Fig. 1).

Overall, after 8 weeks of acclimation to D:L cycle, NA induced a much smaller increase in T_{BAT} than in animals acclimated to L:D cycle. Daily variations were also recorded. The largest increase in T_{BAT1} after NA injection was again recorded at the end of the day (CT 8), while the smallest increase was recorded at the end of night (CT 20). After the injections at CT 4, CT 8, CT 12 and CT 20, the increase in T_{BAT1} after NA was much smaller than that in hamsters acclimated to L:D cycle ($P<0.001$, 0.05, 0.001 and 0.001, respectively; Fig. 1). After 8 weeks in D:L cycle, the response to saline was also smaller than in L:D cycle at CT 0 ($P<0.01$), CT 4 ($P<0.001$), CT 8 ($P<0.01$), CT 12 ($P<0.01$) and CT 20 ($P<0.05$).

Preferred ambient temperature (PT_a; Fig. 2)

After 8 weeks of acclimation to D:L cycle, the daily rhythm of PT_a was also re-entrained (three-way ANOVA: $F_{(5,93)}=2.49$, $P<0.04$; Fig. 2). Before injection, PT_a of saline- and NA-injected animals did not differ. Changes in PT_a after injection were closely correlated with type of injection, photoschedule and time of day (three-way ANOVA: $F_{(5,100)}=3.62$, $P<0.005$; Fig. 2). In D:L cycle, NA injection at the beginning of night (CT 12) induced the largest decrease of PT_a, to a similar degree as that in L:D cycle. After other NA injections, changes in preferred ambient temperatures were smaller than in L:D cycle ($P<0.001$). After 8 weeks under the reversed photoschedule, saline injections induced significant changes in PT_a only at CT 0 and CT 12 (Fig. 2). Significant differences in PT_a changes after saline injections, between hamsters acclimated to L:D and D:L cycles, were recorded at CT 0 ($P<0.001$), CT 4 ($P<0.04$), CT 12 ($P<0.001$) and CT 20 ($P<0.001$).

Correlation between T_{BAT0}, T_{BAT1} and (PT_a; Table 1)

In the hamsters acclimated to D:L cycles for 8 weeks, there was a strong inverse correlation between T_{BAT1} and T_{BAT0} (Table 1). T_{BAT0} was also inversely correlated with PT_a ($r=-0.63$, $P<0.001$). The increase in T_{BAT1} induced by saline injections was correlated with a decrease in PT_a ($r=0.38$, $P<0.001$). In NA-injected animals, such a correlation was not recorded.

Influence of photoschedule and total time of acclimation to 12 hr of light on T_{BAT1} and PT_a after saline and NA injections (Fig. 3)

Changes in BAT temperature after injection (T_{BAT1}) during the 12 weeks of experiments were significantly correlated with photoschedule and type of injection (two-way ANOVA: $F_{(2,176)}=9.84$, $P<0.04$; Fig. 3A). The mean increase in T_{BAT1} after NA injections in L:D cycle (mean for all injections $0.9\pm0.1^{\circ}C$) was larger than that in D:L cycle, after both 4 and 8 weeks of acclimation ($0.5\pm0.1^{\circ}C$, $P<0.001$ and $0.3\pm0.03^{\circ}C$, $P<0.001$; respectively). In saline-injected hamsters acclimated to L:D cycle, mean increase in T_{BAT1} ($0.3\pm0.1^{\circ}C$) was similar to the increase recorded in animals acclimated to D:L cycle for 4 weeks ($0.2\pm0.04^{\circ}C$). In both experiments, changes in

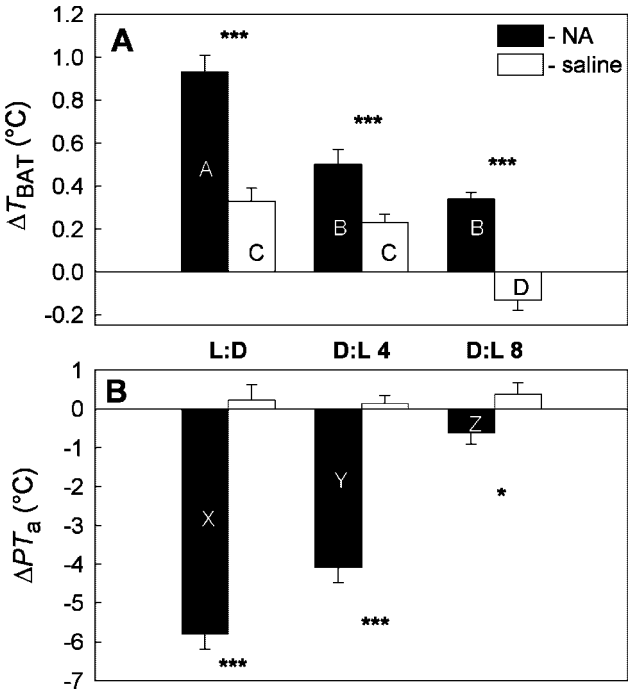

Fig. 3. Mean (mean for all injections ± SE) increase in brown adipose tissue temperature (ΔT_{BAT}; panel **A**) and mean decrease in preferred ambient temperature (ΔPT_a; panel **B**) after noradrenaline (NA) and saline injections in Siberian hamsters acclimated for 4 weeks L:D cycle (L:D) and then after 4 and 8 weeks of acclimation to a reversed photoschedule (D:L 4 and D:L 8, respectively). Stars indicate significant differences between saline- and NA-injected hamsters: $^{*}P<0.05$, $^{***}P<0.001$. The response to NA gradually lowered with the time of acclimation. Significantly different values within each group are indicated by the superscripts: A–B, C–D, X–Y, X–Z, Y–Z: $P<0.001$.

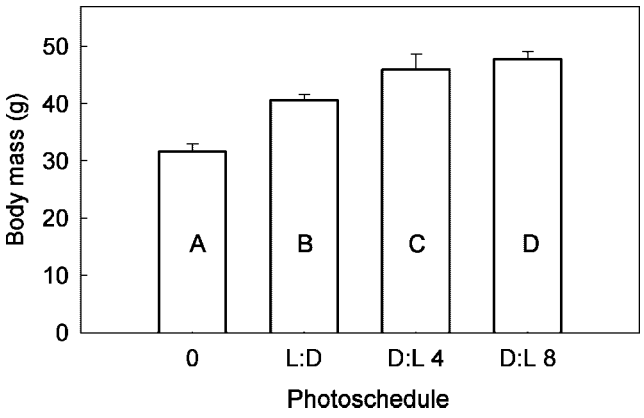

Fig. 4. Mean body mass (±SE) of Siberian hamsters before acclimation (0), after 4 weeks of acclimation to L:D cycle (L:D) and then after 4 and 8 weeks of acclimation to a reversed photoschedule (D:L 4 and D:L 8, respectively). Body mass gradually increased with the time of acclimation. Significantly different values are indicated by the superscripts: A–C, A–D: $P<0.001$, A–B: $P<0.02$, B–D: $P<0.05$.

T_{BAT1} were larger than after 8 weeks in D:L cycle (-0.1 ± 0.05°C, $P<0.001$).

Changes in mean preferred ambient temperature (PT_a) were also closely correlated with photoschedule and type of injection (two-way ANOVA: $F_{(2,176)}=8.84$, $P<0.0002$; Fig. 3B). Changes in mean PT_a after saline administration did not differ between experiments (0.2 ± 0.4°C in L:D cycle, 0.15 ± 0.2°C in D:L cycle—4 weeks, and 0.4 ± 0.3°C in D:L cycle—8 weeks, $P=0.97$). In NA-injected hamsters, the largest decrease in PT_a was recorded in L:D cycle (mean for all injections: -5.8 ± 0.4°C); it was larger than the mean decrease in D:L cycle, after 4 and 8 weeks of acclimation (-4.1 ± 0.4°C, $P<0.001$ and -0.6 ± 0.3°C, $P<0.001$; respectively). The difference between mean changes in PT_a after NA injections in hamsters acclimated to D:L cycle for 4 and 8 weeks was also significant ($P<0.001$).

Changes in body mass (m_b; Fig. 4)

Before acclimation to L:D cycle, mean m_b of the hamsters was 31.6 ± 1.4 g ($n=9$) and increased significantly with the time of acclimation (one-way ANOVA: $F_{(3,20)}=14.36$, $P<0.0001$; Fig. 4). After 4 weeks of acclimation to L:D cycle, m_b increased to 40.6 ± 1.0 g ($P<0.02$), and after 4 weeks in D:L cycle, to 45.9 ± 2.8 g ($P<0.001$). There was no significant difference between m_b of hamsters acclimated to L:D and D:L cycles (4 weeks) ($P=0.25$). After 8 weeks under a reversed photoschedule, m_b increased to 47.7 ± 1.4 g; hamsters were considerably heavier than before acclimation ($P<0.001$) and after acclimation to L:D cycle ($P<0.05$).

DISCUSSION

Twelve weeks of exposure of hamsters to 12 hr of light (irrespective of photoschedule reversal) led to the gradual attenuation of regulatory NST. The mean increase in BAT temperature (T_{BAT1}) and mean decrease in preferred ambient temperature (PT_a), recorded after NA injections, were largest after 4 weeks of acclimation to L:D cycle and least after 8 weeks of acclimation to D:L cycle. One might relate this reaction to the habituation to repeated injections. However, this is not the case because there was no difference in the response to injections between hamsters used for the first time and those re-tested. Furthermore, hamsters acclimated to cold and a short photoperiod for

6 months, and then injected with NA and saline every 4 weeks did not exhibit such a gradual decrease in BAT capacity for NST (Jefimow et al., 2004b).

The gradual decrease in BAT capacity for NST observed in the hamsters seems to be a natural consequence of the photoperiodic history of the animals. For the Siberian hamster, a 12L:12D photoperiod was regarded as short or intermediate, between long and short ones and vice versa (Hoffmann, '73). The present experiments began in March, when the natural photoperiod was about 12L:12D. Despite the additional 12 weeks of exposure to 12 hr of light, hamsters changed to their summer status characterized by low capacity for NST, grey fur and increased body mass (Hoffmann, '73; Heldmaier et al., '82). In the present experiments, the hamsters significantly increased body mass with the time of acclimation ($P < 0.0001$) and at the end of experiments, they were about 30% heavier than at the beginning. Concomitantly NST capacity decreased. These two facts clearly indicate that at the time of experiments hamsters were photorefractory and that they possessed a memory of the previous photoperiodic conditions. Despite prolonged exposure to 12 hr of light and photoschedule reversal, the hamsters not only continued to change to their summer status but they were also able to fully acclimate to the reversed photoschedule: daily rhythms of body temperature, preferred ambient temperature and NST capacity were entrained to the new D:L cycle. Since intermediate photoperiod (12L:12D) occurs in a temperate zone twice a year (in spring and autumn), the direction of changes in day length is very important. Lengthening or shortening photoperiod provides information (encoded in the melatonin signal) about the forthcoming time of year and allows hamsters to prepare for winter or summer conditions. However, critical day length plays a very important role only during the photosensitivity phase, while photorefractoriness is controlled endogenously and does not depend on critical photoperiod (Gorman and Zucker, '98; Goldman, 2001). Photosensitivity in autumn and photorefractoriness in spring have an adaptive value and are very important for photoperiodic mammals in their annual rhythms of reproduction and thermoregulation (Goldman et al., 2004). The Siberian hamsters breed during late spring and summer when food is abundant and ambient temperature is relatively high. On the contrary, a gradual increase in NST capacity and decrease in body mass in autumn ensure winter survival. Taken together, the gradual decrease in the capacity for NST and increase in body mass recorded during this study reflects hamsters' entering summer status despite being housed in constant environment. Physiological, morphological and behavioural changes observed during the time of photorefractoriness cannot be prevented by continuous exposure to a short photoperiod or melatonin infusion (Heldmaier et al., '81; Lynch and Puchalski, '86; Puchalski and Lynch, '86; Schlatt et al., '95; Goldman, 2001; Goldman et al., 2004) or by photoschedule reversal studied in the present experiments.

Siberian hamsters are nocturnal (Scribner and Wynne-Edwards, '94; Ross, '98; Ruf and Heldmaier, 2000). In hamsters acclimated to L:D cycle, a clear-cut rhythm of BAT temperature (T_{BAT0}), with higher values at night, was recorded. After 4 and 8 weeks of acclimation to a reversed photoschedule, the hamsters' daily rhythms of T_{BAT0} and preferred ambient temperature were reversed and entrained to the new conditions. BAT temperature was always lower and preferred ambient temperature was higher by day than by night. The re-entrainment of body temperature (T_b) rhythm after reversal of L:D cycle was also recorded in Wistar rats (Zerath et al., '94). Rhythmic T_b persisted for 2 days after photoperiod reversal, and was fully entrained to the new photoperiod within 7–9 days following reversal. Thus, it is not surprising that after 4 weeks under a reversed photoschedule, BAT temperature rhythm of Siberian hamsters was completely re-entrained to the new D:L cycle.

The results of the present experiments also confirmed our prediction that BAT capacity for regulatory NST depends on the actual level of T_b. In hamsters acclimated to L:D cycle, we recorded large daily variations in the response to NA. The NA-induced increase in BAT temperature (T_{BAT1}) was inversely correlated with BAT temperature before injection (T_{BAT0}); that is, when T_{BAT0} was high, NA elicited a smaller increase in T_{BAT1} ($P < 0.002$). The most pronounced effect of NA was recorded after injection during the day and during the second part of the night. A similar pattern was found in previous studies on Siberian hamsters acclimated to cold ($T_a = 10^\circ C$) and a short photoperiod (8L:16D), when abdominal T_b or preferred ambient temperature were measured (Jefimow et al., 2000, 2003). In the present study, the increase in T_{BAT} after NA administration was also correlated with decrease in PT_a ($P < 0.001$). This

correlation suggests that NA-induced changes in PT_a reflect the intensity of NST in BAT. When the increase in T_{BAT1} is large, animals may choose a lower T_a in order to dissipate excess heat. This preference for a lower T_a may prevent them from overheating, and indicates the importance of behavioural thermoregulation that supports autonomic reactions in maintenance of T_b at the level determined by their set point (Gordon, '93). Selection of lower T_a recorded during the α phase of the 24-hr cycle may prevent an excessive elevation in T_b (Gordon, '90, '94).

We also predicted that daily rhythm of NST would follow daily rhythm of body temperature. However, after 4 weeks of acclimation to a reversed photoschedule, BAT capacity for NST decreased, and rhythmicity of NST capacity disappeared. Moreover, there was no correlation between T_{BAT0} and T_{BAT1} after NA injection. This implies that the NST rhythm is not a direct result of T_b rhythm, although they are related.

Rhythmicity of the response to NA appeared again after 8 weeks of acclimation to D:L cycle. The maximum effects of NA were recorded after the daytime injection (CT 4 and CT 8), when T_{BAT0} was low, similar to what was observed in L:D cycle. Due to the hamsters changing to the summer status, with lowered NST capacity, rhythmicity of NST in BAT was much more difficult to detect. However, a significant inverse correlation between T_{BAT0} and T_{BAT1} after NA injection ($P<0.001$) indicates re-entrainment of the NST rhythm. We are not able to say exactly at which time this re-entrainment was complete, but it occurred between the fifth and eighth week of acclimation to the reversed photoschedule. Thus, the establishment of a new reversed rhythm of NST takes more time than the establishment of a body temperature rhythm. This rhythm seems to be more conservative than T_b rhythm, and it does not seem to be responsible for generation of daily fluctuations in T_b.

A daily rhythm of the response to exogenous NA was found in golden and common spiny mice (Kronfeld et al., '94; Haim and Zisapel, '99). Maximum NST capacity was recorded when body temperature and oxygen consumption were at their circadian minimum. In contrast, in wood mice, the maximum capacity for NST was recorded when body temperature was high (Haim et al., '95). When NST is already activated (e.g., during the α phase), further stimulation with exogenous NA may elicit little effect, since endogenous NA occupies the adrenergic receptors (Kronfeld et al., '94; Jefimow et al., 2003). Daily variations in NST capacity may also result from the daily rhythms of heat production and heat loss since both of them undergo circadian variations, contributing to the generation of the T_b rhythm (Aschoff, '83; Refinetti and Menaker, '92). Since heat loss is promoted during the α phase of every 24-hr cycle (Aschoff, '81), the increase in T_{BAT} after NA injection was smaller at night or when the hamsters were active. These two rhythms, heat production and heat dissipation, as well as daily rhythm of activity might contribute to the daily variations in the response to saline that reflects stress of handling and injection. The magnitude of changes in T_{BAT} after saline might also result from the fact that stress-induced hyperthermia depends on the time of day (Peloso et al., 2002).

We conclude that BAT capacity for NST has a daily rhythm in Siberian hamsters. After photoschedule reversal, the re-entrainment of body temperature (T_b) and preferred ambient temperature (PT_a) rhythms precede re-entrainment of the NST rhythm. NST rhythm seems to have lower plasticity and adjusts to the new environment slower than T_b and PT_a. The present experiments provide evidence for the idea that BAT capacity for NST depends on T_b; that is, there is an inverse correlation between NST capacity and T_b. We suggest that, in Siberian hamsters, during the ρ phase of the day, when T_b is maintained at a lower level and obligatory NST is low, a high capacity for regulatory (BAT-generated) NST might ensure the possibility to rapidly elevate T_b.

To the best of our knowledge, this is the first report describing the hamsters' ability to entrain their rhythms of body temperature, preferred ambient temperature and NST capacity to the reversed photoschedule during the time of photorefractoriness. The present experiments began in March, when hamsters were photorefractory. In the course of acclimation, they continued to change to their summer status and reversal of photoschedule did not prevent gradual, annual changes in body mass and NST capacity. Daily rhythms of body temperature, preferred ambient temperature and NST capacity were fully entrained to the new lighting conditions. These rhythms are determined not only by prevailing photoperiod but also by day length previously experienced. Our results also indicate that photoperiodic history of animals, prior to their use in experiments, is of great importance and must always be taken into account when

studying seasonal rhythms under constant laboratory conditions.

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